AMENDMENTS TO THE DRAWINGS:

The drawings have been amended by replacing FIGS. 1A, 1B, 1C, 1D, 2A, 2B, 3, 4, 5, 6, 7, 8A, 8B, 9A, 9B, 10, 11, and 12 with the enclosed replacement sheets 1-16 containing FIGS. 1A, 1B, 1C, 1D, 1E, 2A, 2B, 3, 4, 5, 6A, 6B, 7, 8A, 8B, 9A, 9B, 10, 11, and 12. Replacement Sheets 1-16 have been properly labeled "REPLACEMENT SHEETS" in compliance with 37 C.F.R. § 1.121(d).

Replacement sheets 1-5 contain FIGS. 1A, 1B, 1C, 1D and 1E , respectively, which show enlarged sequences, replacing original sheets 1-5 which contained FIGS. 1A, 1B, 1C, 1D and 2A, respectively. FIG. 1E is a continuation of FIGS. 1A, 1B, 1C and 1D, which contain the sequences as disclosed in the original FIGS. 1A, 1B, 1C and 1D. Replacement sheet 6 contains FIG. 2A, replacing original sheet 6 which contained FIG. 2B. Replacement sheet 7 contains FIG. 2B, replacing original sheet 7 which contained FIG. 3. Replacement sheet 8 contains FIGS. 3 and 4, replacing original sheet 8 which contained FIG. 4. The two drawings of replacement sheet 10 have been labeled FIGS. 6A and 6B, respectively, replacing the label FIG. 6 for both drawings in the original sheet 10.

All drawings have now complied with the requirements of 37 C.F.R. § 1.84. No new matter has been introduced by these amendments.

REMARKS

Claims 1-14 and 23-28 are pending after entry of the present amendment. Claims 1, 5, 6, 11, 12, 13, and 14 have been amended. Claims 15-22 have been withdrawn. Claims 15, 17, 19-22 although withdrawn have been amended. New claims 23-31 have been added. Applicants reserve the right to prosecute withdrawn or canceled subject matter in one or more related applications. The new claims and amendments are supported by the originally filed specification and claims. Specifically, the new claims and amendment are supported, *inter alia*, at paragraphs [0017], [0021], [0023], [0024], [0025], [0035], [0043], [0045], [0046], [0058], [0118], [0166], and [0208]. No new matter has been added. Applicants believe that all objections and rejections have been obviated and the application is now in condition for allowance.

I. FORMAL OBJECTIONS

(a) SPECIFICATION:

The Examiner objected to the specification allegedly because trademarks are disclosed throughout the instant specification and not all of them are capitalized or accompanied by the generic terminology. Applicants have reviewed the specification and have amended the specification pursuant to the Examiner's recommendations. All trademark and registered names have been capitalized and accompanied by their respective generic terminologies, if available. Accordingly, this objection has been obviated.

Contrary to the Examiner's assertion, there is no registered trademark "TWEEN-80®". Therefore, the term "tween 80" remains in the specification.

Applicants have amended paragraph [0019] of the Publication to change the reference of FIG. 6 to FIGS. 6A and 6B, respectively to conform with the description of FIGS. 6A and 6B in the originally filed specification.

Applicants have amended paragraph [0030] of the Publication to change the reference of FIGS. 1A-D to FIGS. 1A-E to conform with the amendments to the drawings.

(b) DRAWINGS:

The Examiner objected to FIGS. 3, 4, 5, 7, 8, and 9 as being dark. Applicants have replaced all figures (FIGS. 1A, 1B, 1C, 1D, 2A, 2B, 3, 4, 5, 6, 7, 8A, 8B, 9A, 9B, 10, 11, and 12 with replacement sheets 1-16, properly labeled in the top margin as "REPLACEMENT SHEETS" in compliance with 37 C.F.R. § 1.121(d). FIG. 1E has been added as a continuation of FIGS. 1A, 1B, 1C and 1D, which contain the sequences as disclosed in the original FIGS. 1A, 1B, 1C and 1D. FIG. 6 has been replaced with a replacement sheet of FIGS. 6A and 6B to conform with the description of the figures in the originally filed specification. All drawings have now complied with the requirements of 37 C.F.R. § 1.84. No new matter has been added by these amendments.

(c) SEQUENCE LISTING:

The Examiner objected to the disclosure allegedly due to Applicants' non-compliance of the formal requirements of 37 C.F.R. §§ 1.821-825. The Examiner alleged that there is no sequence identifier at page 70, lines 28-29 of the specification (page 28, paragraph [0232] of the Publication). Applicants submit that "EGCG" is not a sequence. "EGCG" stands for "epigallocatechin gallate", an antioxidant, and therefore, does not require a sequence identifier.

(d) CLAIM OBJECTIONS:

The Examiner objected to claims 5-6 and 12-13 because the claims depend from a rejected based claim. It is respectfully submitted that this objection has been obviated by amended the claims as independent claims. As such, claims 5-6, and 12-13 are allowable.

II. THE REJECTIONS UNDER 35 U.S.C. § 112, SHOULD BE WITHDRAWN

Claims 1-4, 7-11, 14 and 23 are rejected under 35 U.S.C. § 112, first paragraph, for allegedly containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors, at the time the application was filed, had possession of the claimed invention.

The Examiner alleged that the claimed invention is directed to a modified PAI-1 molecule, that is only defined by functional properties and that there is no indication of the

structure of the modified product as no reference structure is provided in the claims. The Examiner further alleges that without sufficient recitation of identifying characteristics, the claimed genus of polypeptides could include non-functional proteins or proteins with a different function. Although the Applicants disagree with the Examiner's allegation, in order to expedite prosecution, claim 1 is amended to recite that the modified PAI-1 molecule displays one or more functional activities of an unmodified PAI-1 protein and comprises an amino acid sequence that has at least about 80% similarity to SEQ ID NO:2. Furthermore, claim 1 is amended to recite that amino acid residues that do not contain a sulfhydryl group in a corresponding wild-type PAI-1 protein are each substituted by an amino acid residue that contains a sulfhydryl group such that one or more disulfide bridges are formed between or within the helix D region, A3 strand, A4 strand, and/or A5 strand. As such, the claimed product is defined by both structural characteristics and functional properties.

The Examiner alleged that claim 14 is directed to a method of treating and preventing a disease or disorder related to angiogenesis and that the specification describes inhibition and treatment but does not describe prevention. Although the Applicants disagree, in order to expedite prosecution, claim 14 is amended to recite a method of treating a disease or disorder related to aberrant angiogenesis.

The Examiner further alleged that the claimed invention is directed to a genus of proteins having substitutions that are not sulfhydryl groups but there is no indicia as to where the substitutions occur. Claim 1 has been amended to recite that one or more disulfide bridges are formed between or within the helix D region, A3 strand, A4 strand, and/or A5 strand of the PAI-1 molecule. As such, the claim describes distinguishing identifying characteristics sufficient to show that the applicant was in possession of the claims invention. Specifically, the specifcation at paragraphs [0043], [0045], [0046] teaches that mutations are introduced to wild-type PAI-1 protein to form modified PAI-1 molecule such that disulfide bonds are formed between or within helix D and the ß-sheet, which comprises A3, A4 and A5 strands. The specification teaches that when one or more intramolecular bonds are introduced into the PAI-1 protein, the active form of the modified PAI-1 molecule has a longer *in vivo* half-life as compared to an unmodified PAI-1 protein (Publication at page 3, paragraph [0021]). Applicants submit that the claimed invention teaches a correlation between the structure and the function of the modified PAI-1 protein.

The Examiner alleged that the specification does not provide a representative number of species for the claimed genus. Applicants submit that what constitutes a "representative number" is an inverse function of the skill and knowledge in the art. MPEP 2163. Applicants submit that description of a representative number of species does not require the description to be of such specificity that it would provide individual support for each species that the genus embraces. Here, the inventors discovered that by restraining the movement of the A3 strand and/or the A5 strand and/or limiting the flexibility of the helix D region, the *in vivo* half life of the active form of PAI-1 protein is increased (Publication at page 3, paragraph [0023]). Manipulation of the structure of the PAI-1 protein can be accomplished by substituting an amino acid residue that does not have a sulfhydryl group with an amino acid residue that has a sulfhydryl group. Since the skill and knowledge in the art is high, *i.e.*, the locations of the A3 strand, A4 strand, A5 strand and Helix D region are known (Publication at pages 2-3, paragraph [0017]), and the structure of a modified PAI-1 molecule can be predicted using crystallographic analysis, sequence alignment, structure prediction and homology modeling using computer software programs that are available in the art (Publication at page 20, paragraph [0153]), the representative number for a satisfactory disclosure is low.

Applicants submit that the specification teaches seven cysteine-substituted mutants generating possible sites for disulfide bridge formation at various parts of the modified PAI-1 molecule (Publication at page 25, paragraph [0208], Table). Accordingly, the Applicants have provided actual reduction to practice of the present invention. Exemplary cysteine-substituted mutants have disulfide bridge formed at the helix D region, the top part of A3, A5 strand, the bottom part of A3, A5 strand, or a combination thereof. The cysteine-substituted mutants were found to have normal enzymatic activity and increased *in vivo* half life compared to unmodified PAI-1 proteins. One skilled in the art, in view of the teachings in the specification, would recognize that Applicants was in possession of the necessary common attributes or features of the elements possessed by the members of the genus in view of the species disclosed.

Claims 1-4, 7-11, 14, and 23 are rejected under 35 U.S.C. §112, first paragraph, for lack of enablement. The Examiner alleged that the specification, while being enabling for the protein set forth in SEQ ID NO:2 with modifications via substitutions of sulfhydryl groups at

specific positions, does not reasonably provide enablement for any substitutions that are not sulfhydryl groups throughout SEQ ID NO:2 or a method of prevention. Claim 14 has been amended to recite a method of treatment.

The Examiner alleged that since the claims do not recite a structure and that the claims encompass variants that may not have any biological activity, it would require undue experimentation to practice the claimed invention. As discussed above, Applicants submit that claim 1 has been amended to recite that the modified PAI-1 molecule displays one or more functional activities of an unmodified PAI-1 protein and comprises an amino acid sequence which is at least about 80% similarity to SEQ ID NO:2. Furthermore, claim 1 is amended to recite that the one or more disulfide bridges are formed between or within the helix D region, A3, A4, and/or A5 strands. As such, the claimed product is defined by both structural characteristics and functional properties.

Applicants submit that the specification provides sufficient guidance to one skilled in the art to make and use the invention commensurate in scope with the claims. The Examiner alleged that no correlation is made between the structure and function with regard to the recited modification. As discussed above, the present invention is based on the discovery that by restraining the movement of the A3 strand, the A5 strand and/or limiting the flexibility of the helix D region, the *in vivo* half life of the active form of PAI-1 protein is increased. The specification teaches that this can be achieved when one or more intramolecular bonds are introduced into the PAI-1 protein (Publication at page 3, paragraph [0021]). Thus, the specification did provide a correlation between the structure and the function of a modified PAI-1 protein. The specification further teaches that the intramolecular bonds can be introduced by substituting one or more amino acid residues that do not contain a sulfhydryl group with an amino acid residue that contains a sufhydryl group.

The Examiner alleged there is no guidance as to the positions within the protein's sequence where amino acid substitutions can be made with a reasonable expectation of success. The Examiner further alleged that it is not routine in the art to screen large numbers of mutated proteins to find one that functions as described. Contrary to the allegation of the Examiner, Applicants submit that since the positions of the helix D region, A3, A4, and A5 strands are known and the three-dimensional structure of the PAI-1 protein is also known,

one skilled in the art using the teachings of the present invention would recognize the location of the amino acid substitutions with a reasonable expectation of success. Furthermore, instead of screening large number of randomly mutated PAI-1 proteins, only modified PAI-1 molecules comprising amino acid sequence with a predicted structure taught by the present invention need to be screened. Such structure can be predicted using crystallographic analysis, sequence alignment, structure prediction and homology modeling using computer software programs that are available in the art. As discussed above, Applicants submit that the specification teaches working examples of modified PAI-1 molecules (Publication at page 25, paragraph [0208]).

The Examiner alleged that the claims encompass variants that may not have any biological activity. As discussed above, claim 1 has been amended to recite the modified PAI-1 protein possesses one or more unmodified PAI-1 activities. The *in vitro* activities and *in vivo* biological functions of modified PAI-1 molecules can be measured according to the teachings in the specification (Publication at page 20, paragraphs [0159], [0160]; page 25, section 6.3; section 6.4). For example, the ability of a modified PAI-1 molecule to bind an antibody, uPA, or tPA can be measured using various immunoassays (Publication at page 20, paragraphs [0159], [0160]). The ability of a modified PAI-1 molecule to inhibit uPA can be measured by an amidolytic assay (Section 6.3). The half-life of a modified PAI-1 molecule can be determined by any method for measuring PAI-1 levels (Publication at page 21, paragraph [0164]). As such, the specification enabled one skilled in the art to make the claimed invention.

The Examiner alleged that since the claims encompass a wide variability of protein, said protein may not be effective in the treatment method. Applicants submit that the specification teaches the method of using the claimed invention. The specification teaches that modified PAI-1 molecule has an anti-angiogenic activity as determined by sprout formation assay in human umbilican vein endothelial cell aggregates; and chicken chorioallantoic membrane assay (Publication at pages 27-28, Section 6.12). Also, the specification shows that modified PAI-1 reduces tumor progression in a SCID mouse model. Thus, the specification has shown that modified PAI-1 molecules are effective in the claimed treatment method. The specification also provided assays to determine whether a particular modified PAI-1 molecule is effective in treatment. As such, Applicants submit that the

specification provides sufficient guidance to inform a skilled artisan how to make and use the claimed invention.

Claim 14 is rejected under 35 U.S.C. §112, second paragraph, as failing to particularly point out and distinctly claim the subject matter. Applicants disagree with the rejection. However, in order to expedite prosecution, claim 14 is amended to recite a method of treating a disease or disorder related to aberrant angiogensis in a subject.

The Examiner considered the reference Schattauer, 2000, Thromb Haemost, vol. 84: 919-920 ("Schattauer") pertinent to Applicants' disclosure. Applicants submit that Schattauer teaches the effect of the sulfhydryl group in the drug captopril on antithrombotic effect. It does not teach or suggest the present invention.

The Examiner has imposed a restriction requirement on the present application and states that when a product claim is subsequently found allowable, requirement for restriction between the product claims and the rejoined process claims will be withdrawn. Applicants hereby request rejoinder of the process claims 15-22 which depend from or otherwise include all the limitations of the patentable product.

CONCLUSION

In view of the foregoing amendments and remarks, Applicants respectfully submit that all of the formal objections and rejections of the pending claims have been obviated and the rejections should be withdrawn. No new matter has been added by these amendments. Applicants respectfully submit that all claims are now in condition for allowance. Accordingly, allowance of the present application is respectfully requested.

Respectfully submitted,

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